

PUB-NO: EP001186672A2

DOCUMENT-IDENTIFIER: EP 1186672 A2

TITLE: Polymorphisms in the human organic anion transporter C (OATP-C) gene

PUBN-DATE: March 13, 2002

INVENTOR-INFORMATION:

NAME	COUNTRY
<u>ADEOKUN</u> , MONISOLA	GB
AMBROSE, HELEN JEAN	GB
CRESSWELL, CARL JOHN	GB
DUDLEY, ADAM JESTON	US

ASSIGNEE-INFORMATION:

NAME	COUNTRY
ASTRAZENECA AB	SE

APPL-NO: EP01306983
APPL-DATE: August 17, 2001

PRIORITY-DATA: US22690900P (August 23, 2000)

INT-CL (IPC): C12Q 1/68; C07K 14/47; C07K 16/18
EUR-CL (EPC): C07K014/47; C12Q001/68

ABSTRACT:

CHG DATE=20020403 STATUS=0> This invention relates to polymorphisms in the human OATPC gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the OATPC gene, and to the use of OATPC polymorphism in treatment of diseases with OATPC transportable drugs such as statins.

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11)

EP 1 186 672 A2

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
13.03.2002 Bulletin 2002/11

(51) Int Cl.7: **C12Q 1/68**, C07K 14/47,
C07K 16/18

(21) Application number: **01306983.6**

(22) Date of filing: **17.08.2001**

(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE TR**
Designated Extension States:
AL LT LV MK RO SI

- **Ambrose, Helen Jean**
Cheshire, SK10 4TG (GB)
- **Cresswell, Carl John**
Cheshire, SK10 4TG (GB)
- **Dudley, Adam Jeston**
Wilmington, DE 19850-5437 (US)

(30) Priority: **23.08.2000 US 226909 P**

(71) Applicant: **AstraZeneca AB**
151 85 Södertälje (SE)

(74) Representative: **Giles, Allen Frank et al**
AstraZeneca PLC
Global Intellectual Property
Mereside
Alderley Park
Macclesfield, Cheshire SK10 4TG (GB)

(72) Inventors:

- **Adeokun, Monisola**
Cheshire, SK10 4TG (GB)

(54) **Polymorphisms in the human organic anion transporter C (OATP-C) gene**

(57) This invention relates to polymorphisms in the human OATPC gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic var-

iation in the OATPC gene, and to the use of OATPC polymorphism in treatment of diseases with OATPC transportable drugs such as statins.

EP 1 186 672 A2

Description

[0001] This invention relates to polymorphisms in the human OATPC gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the OATPC gene, and to the use of OATPC polymorphism in treatment of diseases with OATPC transportable drugs.

[0002] Na⁺-independent organic anion transporting polypeptide (OATP) C gene is a member of the OATP supergene family involved in multifunctional transport of organic anion. OATPC transports the organic anion taurocholate, conjugated steroids: DHEAS, estradiol 17 β -D-glucuronide and estrone-3-sulfate, eicosanoids: PGE₂, thromboxane B₂, leukotriene C₄, and E₄, and thyroid hormones T₄ and T₃^{1,2}. OATPC has also been shown to be involved in the transport of xenobiotics, and drugs involved in lipid lowering *e.g.* statins¹. Statins have been referred to as a first-line therapy for patients with atherosclerotic vascular diseases. The OATPC gene and its product is also thought to be of importance in other diseases due to its transport of DHEAS an adrenal steroid which has been suggested to have positive neuropsychiatric, immune, and metabolic effects³. Due to the substrate specificity, location in the liver, and being exclusively expressed in the liver, Abe *et al.* suggested that OATPC could be the predominant clearance mechanism of several endogenous and exogenous substrates in the liver. OATPC is the first human molecule reported to transport thyroid hormones².

[0003] This liver specific transporter may be useful in liver-specific drug delivery systems and liver-specific chemotherapy, bile acid formation and the pathogenesis of diseases such as cholestasis, hyperbilirubinemia and thyroid hormone resistance.

[0004] The OATPC gene (sometimes called OAPT2 in the literature) has been cloned by four different groups, annotated and published as EMBL accession numbers AB026257 (OATPC, 2452bp), AF205071 (OATP2, 2830, ref 1), AJ132573 (OATP2, 2778)⁴, and AF060500 (LST-1)². Polymorphism has been reported by Tamai⁵ which is Asn130Asp and Val174Ala although any functional effect was stated therein to be not clear. König (2000) J Biol Chem 275, 23161-23168 describes the genomic organisation of OATP 1, 2 and 8. International patent application WO 00/08157 describes human anion transporter genes and some polymorphisms.

[0005] All positions herein of polymorphisms in the OATPC polynucleotide relate to the position in one of SEQ ID NO 1 or 3-12 unless stated otherwise or apparent from the context.

[0006] All positions herein of polymorphisms in the OATPC polypeptide relate to the position in SEQ ID NO 2 unless stated otherwise or apparent from the context.

[0007] One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics"). Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), Clinical Chemistry, **43**, 254; Marshall (1997), Nature Biotechnology, **15**, 1249; International Patent Application WO 97/40462, Spectra Biomedical; and Schafer *et al.* (1998), Nature Biotechnology, **16**, 33.

[0008] Clinical trials have shown that patient response to treatment with pharmaceuticals is often heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy.

[0009] Point mutations in polypeptides will be referred to as follows: natural amino acid (using 1 or 3 letter nomenclature), position, new amino acid. For (a hypothetical) example "D25K" or "Asp25Lys" means that at position 25 an aspartic acid (D) has been changed to lysine (K). Multiple mutations in one polypeptide will be shown between square brackets with individual mutations separated by commas.

[0010] The present invention is based on the discovery of polymorphisms in OATPC.

[0011] According to one aspect of the present invention there is provided a method for the detection of a polymorphism in OATPC in a human, which method comprises determining the sequence of the human at at least one polymorphic position and determining the status of the human by reference to polymorphism in the OATPC gene. Preferred polymorphic positions are one or more of the following positions:

A method for the detection of a polymorphism in OATPC in a human, which method comprises determining the sequence of the human at at least one of the following polymorphic positions:

positions 510, 696, 1299, 1312, 1347, 1561, 2028, 2327 and 2342 in sequence of the OATPC gene as defined

¹ A Novel Human Hepatic Organic Anion Transporting Polypeptide (OATP2), Hsiang *et al.* J Biol Chem **274**, 37161-37168 (1999)

² Identification of a Novel Gene Family Encoding Human Liver-specific Organic Anion Transporter LST-1, Takaaki Abe *et al.* J Biol Chem **274**, 17159-17163 (1999)

³ Bates *et al.* (1998) Curr. Opin. Endocrinol. Diab. **5**, 357-366

⁴ A novel human organic anion transporting polypeptide localised to the basolateral hepatocyte membrane, König Jorg *et al.* (2000) Am J Physiol. Gastrointest. Liver Physiol. **278**: G156-G164

⁵ Tamai *et al.* (2000), BBRC, **273**, 251-60

EP 1 186 672 A2

by the position in SEQ ID NO: 1;
 positions 400, 405, 488 and 643 in OATPC polypeptide defined by position in SEQ ID NO: 2;
 positions 321 and 1332 defined by position in SEQ ID NO 3;
 position 41 defined by position in SEQ ID NO 4;
 positions 109 and 244 defined by position in SEQ ID NO 5;
 positions 117 and 283 defined by position in SEQ ID NO 6;
 positions 209 and 211 defined by position in SEQ ID NO 7;
 positions 63 to 68 defined by position in SEQ ID NO 8;
 position 53 defined by position in SEQ ID NO 9;
 position 75 defined by position in SEQ ID NO 10;
 position 162 defined by position in SEQ ID NO 11; and
 positions 84 defined by position in SEQ ID NO 12.

[0012] The term human includes both a human having or suspected of having a OATPC mediated disease and an asymptomatic human who may be tested for predisposition or susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

[0013] The term polymorphism includes single nucleotide substitution, nucleotide insertion and nucleotide deletion which in the case of insertion and deletion includes insertion or deletion of one or more nucleotides at a position of a gene.

[0014] The method for diagnosis is preferably one in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

[0015] The status of the individual may be determined by reference to allelic variation at any one, two, three, four, five, six, seven, eight, nine or more positions.

[0016] The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation.

[0017] It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of allelic variation are reviewed by Nollau *et al.*, Clin. Chem. **43**, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Abbreviations:	
ALEX TM	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS TM	Amplification refractory mutation system
b-DNA	Branched DNA
bp	base pair
CMC	Chemical mismatch cleavage
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
FRET	Fluorescence resonance energy transfer
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
LCR	Ligase chain reaction
MASDA	Multiple allele specific diagnostic assay

EP 1 186 672 A2

(continued)

Abbreviations:	
NASBA	Nucleic acid sequence based amplification
OATP	Na ⁺ -independent organic anion transporting polypeptide
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis

Table 1 -

Mutation Detection Techniques

General: DNA sequencing, Sequencing by hybridisation

Scanning: PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

Hybridisation Based

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

Extension Based: ARMS™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

Incorporation Based: Mini-sequencing, APEX

Restriction Enzyme Based: RFLP, Restriction site generating PCR

Ligation Based: OLA

Other: Invader assay

* Note: not useful for detection of promoter polymorphisms.

Table 2 -

Signal Generation or Detection Systems

Fluorescence: FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

Other: Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 -

Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

[0018] Preferred mutation detection techniques include ARMS™, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques.

[0019] Particularly preferred methods include ARMS™ and RFLP based methods. ARMS™ is an especially preferred method.

[0020] In a further aspect, the diagnostic methods of the invention are used to assess the pharmacogenetics of a drug transportable by OATPC.

EP 1 186 672 A2

[0021] Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

[0022] Individuals who carry particular allelic variants of the OATPC gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences arising as a result of allelic variation may have a direct effect on the response of an individual to drug therapy. The diagnostic methods of the invention may be useful both to predict the clinical response to such agents and to determine therapeutic dose.

[0023] In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by OATPC. This may be particularly relevant in the development of hyperlipoproteinemia and cardiovascular disease and the present invention may be used to recognise individuals who are particularly at risk from developing these conditions.

[0024] In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the OATPC gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

[0025] In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes.

[0026] According to another aspect of the present invention there is provided a human OATPC gene or its complementary strand comprising a variant allelic polymorphism at one or more of positions defined herein or a fragment thereof of at least 20 bases comprising at least one novel polymorphism.

[0027] Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

[0028] According to another aspect of the present invention there is provided a polynucleotide comprising at least 20 bases of the human OATPC gene and comprising an allelic variant selected from any one of the following:

Region	variant	Position in SEQ ID NO	SEQ ID NO
Exon 4	A	510	1
Exon 5	T	670	1
Exon 5	T	696	1
Exon 9	G	1299	1
Exon 9	A	1312	1
Exon 9	A	1347	1
Exon 10	C	1561	1
Exon 14	C	2028	1
3'UTR	Insert T	2327	1
3'UTR	C	2342	1
Promoter	G	321	3
Promoter	C	1332	3
Intron 1	A	41	4
Intron 2	G	109	5
Intron 2	G	244	5
Intron 3	A	117	6
Intron 3	A	283	6
Intron 4	A	209	7
Intron 4	A	211	7
Intron 4	Deletion CTTGTA	63	8
Intron 6	T	53	9

EP 1 186 672 A2

(continued)

Region	variant	Position in SEQ ID NO	SEQ ID NO
Intron 9	Insert TTC	75	10
Intron 11	Insert T	162	11
Intron 12	C	84	12

[0029] According to another aspect of the present invention there is provided a human OATPC gene or its complementary strand comprising a polymorphism, preferably corresponding with one or more the positions defined herein or a fragment thereof of at least 20 bases comprising at least one polymorphism.

[0030] Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

[0031] The invention further provides a nucleotide primer which can detect a polymorphism of the invention.

[0032] According to another aspect of the present invention there is provided an allele specific primer capable of detecting a OATPC gene polymorphism, preferably at one or more of the positions as defined herein.

[0033] An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS™ assays. The allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

[0034] An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer.

[0035] Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

[0036] According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a OATPC gene polymorphism, preferably at one or more of the positions defined herein.

[0037] The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

[0038] The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

[0039] According to another aspect of the present invention there is provided an allele specific primer or an allele specific oligonucleotide probe capable of detecting a OATPC gene polymorphism at one of the positions defined herein.

[0040] According to another aspect of the present invention there is provided a diagnostic kit comprising an allele specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.

[0041] The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

[0042] In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphisms of relatively high frequency. The OATPC gene is on chromosome 12p (as shown from a database search with the cDNA as a query sequence). Low frequency polymorphisms may be particularly useful for haplotyping as described below. A haplotype is a set of alleles found at linked polymorphic sites (such as within a gene) on a single (paternal or maternal) chromosome. If recombination within the gene is random, there may be as many as 2ⁿ haplotypes, where 2 is the number of alleles at each SNP and n is the number of SNPs. One approach to identifying mutations or polymorphisms which are correlated with clinical response is to carry out an association study using all the haplotypes that can be identified in the population of interest. The frequency of each haplotype is limited by the frequency of its rarest allele, so that SNPs with low frequency alleles are particularly useful as markers of low frequency haplotypes. As particular mutations or polymorphisms associated with certain clinical features, such as adverse or abnormal events, are likely to be of low frequency within the population, low frequency SNPs may be particularly useful in identifying these mutations (for examples see: Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation

EP 1 186 672 A2

at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* (1998) **62**:481-90, De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, and Gudnason V; and Variation at the von willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* (1999) **93**:4277-83, Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D).

[0043] According to another aspect of the present invention there is provided a computer readable medium comprising at least one novel sequence of the invention stored on the medium. The computer readable medium may be used, for example, in homology searching, mapping, haplotyping, genotyping or pharmacogenetic analysis.

[0044] According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a drug transportable by OATPC in which the method comprises:

i) detection of a polymorphism in OATPC in the human, which detection comprises determining the sequence of the human at one or more of the following positions:

positions 487, 510,, 554, 670, 696, 819, 820, 1299, 1312, 1347, 1561, 2028, 2327 and 2342 in sequence of the OATPC gene as defined by the position in SEQ ID NO: 1;
positions 130, 152, 174, 241, 400, 405, 488 and 643 in OATPC polypeptide defined by position in SEQ ID NO: 2;
positions 321 and 1332 defined by position in SEQ ID NO 3;
position 41 defined by position in SEQ ID NO 4;
positions 109 and 244 defined by position in SEQ ID NO 5;
positions 117 and 283 defined by position in SEQ ID NO 6;
positions 209 and 211 defined by position in SEQ ID NO 7;
positions 63 to 68 defined by position in SEQ ID NO 8;
position 53 defined by position in SEQ ID NO 9;
position 75 defined by position in SEQ ID NO 10;
position 162 defined by position in SEQ ID NO 11; and
positions 84 defined by position in SEQ ID NO 12.

and determining the status of the human by reference to polymorphism in the OATPC gene; and

ii) administering an effective amount of the drug.

[0045] Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which statin drug or drugs to administer and/or in deciding on the effective amount of the statin drug or drugs. Statins already approved for use in humans include atorvastatin, cerivastatin, fluvastatin, pravastatin and simvastatin. The reader is referred to the following references for further information: *Drugs and Therapy Perspectives* (12th May 1997), **9**: 1-6; Chong (1997) *Pharmacotherapy* **17**: 1157-1177; Kellick (1997) *Formulary* **32**: 352; Kathawala (1991) *Medicinal Research Reviews*, **11**: 121-146; Jahng (1995) *Drugs of the Future* **20**: 387-404, and *Current Opinion in Lipidology*, (1997), **8**, 362 - 368. A preferred statin drug is compound 3a (S-4522) in Watanabe (1997) *Bioorganic and Medicinal Chemistry* **5**: 437-444; now called rosuvastatin, see Olsson (2001) *American Journal of Cardiology*, **87**, supplement 1, 33-36. The term "drug transportable by OATPC" means that transport by OATPC in humans is an important part of a drug exerting its pharmaceutical effect in man. For example, some statins have to be transported to the liver by OATPC to exert their lipid lowering effects.

[0046] According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a drug transportable by OATPC in which the method comprises:

i) diagnosis of a single nucleotide polymorphism in OATPC gene in the human, which diagnosis preferably comprises determining the sequence of the nucleic acid at position 487 in the coding sequence of the OATPC gene as defined by the position in SEQ ID NO: 1.

and determining the status of the human by reference to polymorphism in the OATPC gene; and

ii) administering an effective amount of the

[0047] Although the polymorphism at position 487 (A→G; Asn130Asp) was reported by Tamai, no functional effect was attributed. Indeed the following polymorphisms are known but have not previously been attributed a role in statin pharmacogenetics as disclosed herein.

OATPC polymorphisms

Position of nucleotide as in sequence 1 of OATPC patent

(continued)

OATPC polymorphisms					
Position of amino acid as in sequence 2 of OATPC patent					
Exon	nucleotide	SNP	Aminoacid	Change	Change
4	487	A>G	130	Asn>Asp	N>D
4	554	G>A	152	Arg>Lys	R>K
5	620	T>C	174	Val>Ala	V>A
5	670	C>T	191	Leu	L
6	819	A>T	240	Val	V
6	820	G>A	241	Asp>Asn	D>N

[0048] According to another aspect of the present invention there is provided use of a drug transportable by OATPC in preparation of a medicament for treating a disease in a human diagnosed as having a polymorphism therein, preferably at one or more of the positions defined herein. Preferably the disease is cardiovascular.

[0049] According to another aspect of the present invention there is provided a pharmaceutical pack comprising OATPC transportable drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism therein, preferably at one or more of the positions defined herein.

[0050] According to another aspect of the present invention there is provided an allelic variant of human OATPC polypeptide comprising at least one of the following:

- a leucine at position 400 of SEQ ID NO 2;
- an isoleucine at position 405 of SEQ ID NO 2;
- an arginine at position 488 of SEQ ID NO 2;
- a phenylalanine at position 643 of SEQ ID NO 2;

or a fragment thereof comprising at least 10 amino acids provided that the fragment comprises at least one allelic variant.

[0051] Fragments of polypeptide are at least 10 amino acids, more preferably at least 15 amino acids, more preferably at least 20 amino acids.

[0052] According to another aspect of the present invention there is provided an antibody specific for an allelic variant of human OATPC polypeptide as described herein.

[0053] Antibodies can be prepared using any suitable method. For example, purified polypeptide may be utilized to prepare specific antibodies. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, and the various types of antibody constructs such as for example F(ab')₂, Fab and single chain Fv. Antibodies are defined to be specifically binding if they bind the allelic variant of OATPC with a K_a of greater than or equal to about 10⁷ M⁻¹. Affinity of binding can be determined using conventional techniques, for example those described by Scatchard et al., *Ann. N.Y. Acad. Sci.*, 51:660(1949).

[0054] Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice or rats, using procedures that are well-known in the art. In general, antigen is administered to the host animal typically through parenteral injection. The immunogenicity of antigen may be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity to antigen. Examples of various assays useful for such determination include those described in: *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radioimmunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530.

[0055] Monoclonal antibodies may be readily prepared using well-known procedures, see for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439 and 4,411,993; *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.), (1980).

[0056] The monoclonal antibodies of the invention can be produced using alternative techniques, such as those described by Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3: 1-9 (1990) which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7: 394 (1989).

EP 1 186 672 A2

[0057] Once isolated and purified, the antibodies may be used to detect the presence of antigen in a sample using established assay protocols, see for example "A Practical Guide to ELISA" by D. M. Kemeny, Pergamon Press, Oxford, England.

[0058] According to another aspect of the invention there is provided a diagnostic kit comprising an antibody of the invention.

[0059] The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

[0060] In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

[0061] AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

[0062] General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

[0063] Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

Example 1

Identification of Polymorphisms

1. Methods

DNA Preparation

[0064] DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

Template Preparation

[0065] Templates were prepared by PCR using the oligonucleotide primers and annealing temperatures set out below. The extension temperature was 72° and denaturation temperature 94°. Generally 50 ng of genomic DNA was used in each reaction and subjected to 35 cycles of PCR. Where described below, the primary fragment was diluted 1/100 and two microlitres were used as template for amplification of secondary fragments. PCR was performed in two stages (primary fragment then secondary fragment) to ensure specific amplification of the desired target sequence.

Polymorphisms in OATPC: cDNA screening of 15 Liver samples				
Region	SNP	Position	Amino Acid Change	Allele frequencies
Exon 4	G/A	510	None	G=96.7% A=3.3%
Exon 5	C/T	670	None	C=50% T=50%
Exon 5	C/T	696	None	C=60% T=40%
Exon 9	C/G	1299	Phe400Leu	C=96.7% G=3.3%
Exon 9	G/A	1312	Val405Ile	G=96.7% A=3.3%
Exon 9	G/A	1347	None	G=96.7% A=3.3%
Exon 10	G/C	1561	Gly488Arg	G=96.7% C=3.3%

EP 1 186 672 A2

(continued)

Polymorphisms in OATPC: cDNA screening of 15 Liver samples				
Region	SNP	Position	Amino Acid Change	Allele frequencies
Exon 14	A/C	2028	Leu643Phe	A=90% C=10%

[0066] OATP2 above refers to the clone sequenced by Hsiang et al (ref 1). Some comment on the numbering of exons in OATPC is required. This gene contains an exon (38 bp) upstream (5' UTR region) of the exon containing the ATG start site for translation. Therefore the exon numbering could vary depending whether this exon is counted as the first exon or not. In the literature, Konig (2000) JBC 275: 23161-68, have defined exon 1 as that containing the ATG start site and therefore we have adopted the same numbering in this application (but note that the priority document relating to the present application did vice versa; for example, exon 5 in this application is equivalent to exon 6 in the priority document).

PCR PRODUCTS		
Fragment	Forward Oligo	Reverse Oligo
443-999	443-466	979-999
874-1360	874-896	1337-1360
1255-1684	1255-1278	1663-1684
1559-2095	1559-1581	2073-2095

RFLP analysis			
Polymorphism	Position	RFLP Enzyme/PCR size	RFLP fragment size
G/A	510		
C/T	670	BmR I/595bp	C=349bp, 246bp T=595bp
C/T	696		
C/G	1299	Apo I/ 595bp	C=30bp, 60bp, 380bp G=90bp, 380bp
G/A	1312	Bst 4Cl/ 595bp	G=77bp, 393bp A=470bp
G/A	1347		
G/C	1561	HpyCH4IV/595bp	G=470bp C=144bp, 326bp
A/C	2028	Ase I/ 577bp	A=89bp,488bp C=577bp

EP 1 186 672 A2

Example 2

[0067]

Further OATPC Polymorphisms							
SNPs in OATPC 3'UTR (positions according to SEQ ID NO 1)							
Exon	Nucleotide	SNP				Frequency	Frequency
						Caucasian	Japanese
3' UTR	2327	Ins T				not screened	0.1
3' UTR	2342	T>C				not screened	0.4
SNPs in OATPC promoter (positions according to SEQ ID NO 3)							
	Nucleotide	SNP				Frequency	Frequency
						Caucasian	Japanese
	321	T>G				0.03	not screened
	1332	A>C				0.08	not screened
SNPs in OATPC introns							
Intron	Nucleotide	SNP	Nucleotide	Sequence			
	position in		position	ID No			
	relation to exon		in sequence				
1	IVS1+21	T>A	41	4			
2	IVS2+89	T>G	109	5			
2	IVS2+224	A>G	244	5			
3	IVS3+97	C>A	117	6			
3	IVS3+263	G>A	283	6			
4	IVS4+189	G>A	209	7			
4	IVS4+191	G>A	211	7			
4	IVS5-118	delCTTGTA	63	8			
6	IVS6+33	C>T	53	9			
9	IVS10-107	ins TTC	75	10			
11	IVS11+142	Ins T	162	11			
12	IVS13-97	G>C	84	12			

EP 1 186 672 A2

OATPC intronic SNPs

Key

- 5 **[0068]** 20bp of exon sequence shown in uppercase
Intron sequence in lowercase (200 to 300bp only)
SNP shown in uppercase (one allele only)

10 **Sequence ID No 4**
IVS1+21 T>A SNP at position 41 in this sequence

GATACTGCAA TGGATTGAAG gtagaataag ttttatgttt Ttgagctaaa ataagtaaat 60
agggaaacttt aatgtataga aaagcaagtt gttaaaaaga acattatgtt tcaaattata 120
15 attttcaatt gaagcatata ttgaaatatt aacataatga ttcatactt gatttaaacc 180
agtcttttaa tctgattaag 200

20 **Sequence ID No 5**
IVS2+89 T>G SNP at position 109 in this sequence
IVS2+224 A>G SNP at position 244 in this sequence

TGACGGAAGC TTTGAAATTG gtaacattta ttttctattt taataaccaa acttgcaaag 60
ttaaaaaata tatatgcttt acaccactgg ttatcaactg gggtaaatTt atctctcaca 120
25 ggcaatttgg caataactaa aaacatttgt ggttgtcata actgcacagg ggttgggggc 180
aatggaagtg ctactgggtat ctaaaggtag aggtcagggg tactgctaaa tattctataa 240
tgcAcaaaga atgatgtaac tgaaaatgtt gatagtgagg atgttcagaa accctgattc 300

30 **Sequence ID No 6**
IVS3+97 C>A SNP at position 117 in this sequence
IVS3+263 G>A SNP at position 283 in this sequence

35

CCACATTTCT TCATGGGATA gtaagtgtta aaaaaaaaaa aaacctctgt gccactatca 60
gtaccttgta aattaggagt agaattttat tattatccct ttaaattaggc agttacCttt 120
40 tgagaagata ccactaagt gtgtacagaa atgaaatagt gtctatttgt ctacataatc 180
attttattta tcgtagcttt catatacttt gaaataacaa aaagactaaa ctgtagagtt 240
tcaaataaaa taaataggct ttttatgaat ttttagtata acGtatatac tgtacgtctt 300

45 **Sequence ID No 7**
IVS4+189 G>A SNP at position 209 in this sequence
IVS4+191 G>A SNP at position 211 in this sequence

50 ACCTGAGATA GTGGGAAAAG gtaagaatta atattgacag taaaaagtct tctaaaatgt 60
atacatTTaa ttacatctct aaaaattgtt gtgatattca ttagcaaaaat ttaattaaga 120
atgaatagga aaaacatttg actcttacag acataattat agtgtaata tacacagttc 180
gccattaac aacacagggt taaactacGc Gttttcactt ctatgcaaat tttgtccatc 240
tgaactggat gataaacctg ccggaagaa tatctgacat tttctatatt tggattgaac 300

55

EP 1 186 672 A2

Sequence ID No 8

IVS5-118 delCTTGTA deletion of 6bp from position 63 to 68 incl.

```

5 tagcagcata agaatggact aatacaccat attgtcaaag tttgcaaagt gaatataaat 60
  taCTTGTAAct tgtaaattaa aaaaaataa gtagaataat taagagttaa caagtagtta 120
  aatttgtaat agaaatgcta aaattaatgt ttaaaatgaa acactctctt atctacatag 180
  GTTGTTTAAA GGAATCTGGG 200

```

10

Sequence ID No 9 IVS6+33 C>T

SNP at position 53 in this sequence

```

TATTGGATAT GTAGATCTAA gtaagtacaa ccagaacaag gtacatgat aaCgtctttc 60
taagcacaca tgcgaaaaac attttttcaa ataactgaat tcaactcttc aatagtcctt 120
tgcttaatat aattagaaaag ttacaagtag gaaataaatg tattactaat cagaataaat 180
ataaaaatcca gctcctattt 200

```

15

Sequence ID No 10

IVS10-107 Ins TTC SNP at position 75 in this sequence

```

20 ttaaaaaaaaa ctttgccatt tgcgtcatcat caaagcaaat ttcttcatat aaagaaaaat 60
  tctttatcta cttt(TTC)ttttcc ctctttctct gctttcactt tacttcttcc ttctcctccc 120
  ctcttttgtc tttttcttct ctctctctct ttttgatata tgcctatcat atatttccag 180
  AAATAATCCA GTGACATCTC 200

```

25

Sequence ID No 11

IVS11+142 InsT InsT at position 162 in this sequence

```

CATGTCATGC TGATTGTTAA gtaagtatga cttttaaaaa cattttcata tgcattgagac 60
tataaacaca cctaattgata tgcataattt tacataatat actgggaatt caaattcata 120
tttcatcaaa ttttaatttt ctgagaattc attttattaa aa(T)ttactatg aactctcaag 180
gctgtaatta ataattttgc 200

```

30

35

Sequence ID No 12

IVS13-97 G>C SNP at position 84 in this sequence

```

tgatttgggt ctttgagatt tctaataatc tttattattg ggtagatgca gaacaaaata 60
ataaacgaat cctccaaatt tttGaacttt tatttaataca aaatataatca atgtggaata 120
tcatgcagtt acatttaaaa tatgttcctt aaactgacat cttctcttct cctattacag 180
GAGGAATTCT AGCTCCAATA 200

```

40

OATPC promoter region

45

[0069] Total length of the sequence = 1538bp

1500bp of OATPC sequence directly upstream from the cDNA sequence

Sequence in uppercase represents 38bp overlap with the cDNA sequence (SEQ ID NO 1) where this 38bp is 5'UTR sequence.

Nucleotide positions in the promoter have been determined where the -1 position is the base (lowercase) directly upstream of the end of the cDNA sequence.

50

55

EP 1 186 672 A2

	atgctcttttg	acctctgaaa	atattggaga	atttttacaac	tggcaccttt	agctcaggat	60
	tataaagggtt	gttagttagt	ttgtactggt	ttatcttcat	tgtatataat	atatatatta	120
	gtctccaaac	atggtgatgt	gttttcaatg	aaatggatgt	ctgaggagaa	aaccattagc	180
5	ctgagaaaaac	ccaaactgta	ttcccatgtt	gaataaaaagg	aagtccataa	aatgatgga	240
	aaatgttctg	cattctgtgt	atgatatcaa	aatctggcag	tacatgaaaa	tttttcaaag	300
	tgcattattta	acaggcataa	tcttttggtct	cctgagccag	aatctgctgg	gtatgggact	360
	ggattgctat	tttgacaact	cgccagtaga	ttcttactca	gcagagtatt	tggaagcctt	420
	actctaatat	tttggccttg	ggtctacatt	tctcagttct	gcacagtcac	tcttcccctc	480
	tacactactc	tttagtttgt	ctcatgattc	caatactctc	aataattaac	caagaataga	540
	actaatcaat	cagataactg	tggcacagac	atcaaataca	ttttgctgca	accatatcaa	600
10	caaatgtccc	atgaatgata	aggggtaacc	atattctcat	atatgcatcc	tcacattacc	660
	acatatatat	atgtgcatat	gtgtatacag	gtaaaaagtgt	gtatatatgt	atacatgtat	720
	gtttgtgtgt	atatacatat	atatactctc	acacttttct	gaaatatata	tattttatgtg	780
	agagaagggt	ctgtacttta	tttcagaaga	gagcttaatg	tccaagggtat	aattgagagt	840
	ctaaaaatggt	tgagttattg	aattaattaa	acttcatctc	tactcaagaa	aacttttaac	900
	tgagttaagc	tcttcctttc	tccacaagtc	aagtcaataa	aaggaaactg	tgatattaat	960
15	aattctttcc	tgttttgatg	taaagaatct	atcgcataaa	gcagtcttaa	ttttcatcat	1020
	tcagaaaaat	ggctcttgag	ttaattggga	ctctcttatt	ccaggtggta	tctccagtct	1080
	ccatacatat	cacgttagaa	ccatacttat	gtaccaagca	aagagggtat	attttaattt	1140
	ttaaatgcca	atgtaacctg	taggcatatt	ttttattttg	cttaaatatt	ttcctatttg	1200
	gaagttttaa	atacctggaa	taattttattg	tactcatatt	tttaaagaaa	aaaatcttat	1260
	gccaccaact	taattgaata	aacaagttaa	agccattccc	aaaagtaagg	tttacttggt	1320
20	aagattaaca	aaaaataatg	tgagaattct	gagaaatata	atctttaaat	attggcaact	1380
	ggagtgaact	cttaaaaacta	actaggtttt	atatgtttga	ctagagcaat	gacataataa	1440
	ggtgggttaat	catcactgga	cttgttttca	aaaagccaac	tactttaaga	ggaataaagg	1500
	GTGGACTTGT	TGCAGTTGCT	GTAGGATTCT	AAATCCAG	1538		
25							
30							
35							
40							
45							
50							
55							

EP 1 186 672 A2

SEQUENCE LISTING

<110> AstraZeneca AB

<120> Chemical Compounds

<130> adeokun
ambrose
cresswell
dudley

<140>

<141>

<160> 12

<170> PatentIn Ver. 2.1

<210> 1

<211> 2452

<212> DNA

<213> Homo sapiens

<400> 1

```

gtggacttgt tgcagttgct gtaggattct aaatccaggt gattgtttca aactgagcat 60
caacaacaaa aacatttcta tgatatctat atttcaatca tggacacaaa tcaacatttg 120
aataaaaacag cagaggcaca accttcagag aataagaaaa caagatactg caatggattg 180
aagatgttct tggcagctct gtcactcagc tttattgcta agacactagg tgcaattatt 240
atgaaaagtt ccatcattca tatagaacgg agatttgaga tatcctcttc tcttgttggt 300
tttattgacg gaagctttga aattggaaat ttgcttctga ttgtatttgt gagttacttt 360
ggatccaaac tacatagacc aaagttaatt ggaatcgggt gtttcattat ggggaattgga 420
gggtgttttga ctgctttgcc acatttcttc atgggatatt acagggtattc taaagaaact 480
aatatcaatt catcagaaaa ttcaacatcg accttatcca ctgttttaat taatcaaat 540
ttatcactca atagagcacc acctgagata gtgggaaaaag gttgttttaa ggaatctggg 600
tcatacatgt ggatatatgt gttcatgggt aatatgcttc gtggaatagg ggagactccc 660
atagtaccac tggggccttc ttacattgat gatttcgcta aagaaggaca ttctctcttg 720
tatttaggta tattgaatgc aatagcaatg attgggtccaa tcattggctt taccctggga 780
tctctgtttt ctaaaatgta cgtggatatt ggatatgtag atctaagcac tatcaggata 840
actcctactg attctcgatg ggttggagct tgggtggctta atttccttgt gtctggacta 900
ttctccatta tttcttccat accattcttt ttcttgcccc aaactccaaa taaaccacaa 960
aaagaaagaa aagcttcact gtctttgcat gtgctggaaa caaatgatga aaaggatcaa 1020
acagctaat ttgaccaatca aggaaaaaat attacacaaa atgtgactgg tttttccag 1080
tcttttaaaa gcacccctac taatccccctg tatgttatgt ttgtgctttt gacgttggtt 1140
caagtaagca gctataatgg tgcttttact tatgtcttca aatacgtaga gcaacagtat 1200
ggtcagcctt catctaaggc taacatctta ttgggagtca taaccatacc tatttttgca 1260
agtgggaattg ttttagggagg atatatcatt aaaaaattca aactgaacac cgttggaatt 1320
gccaaattct catgttttac tgctgtgatg tcattgtcct tttacctatt atattttttc 1380
atactctgtg aaaaacaaac agttgcccga ctaaacatga cctatgatgg aaataatcca 1440
gtgacatctc atagagatgt accactttct tattgcaact cagactgcaa ttgtgatgaa 1500
agtcaatggg aaccagctcg tggaaacaat ggaataactt acatctcacc ctgtctagca 1560
ggttgcaaat ctccaagtgg caataaaaag cctatagtgt tttacaactg cagttgtttg 1620
gaagtaactg gtctccagaa cagaaattac tcagcccat tgggtgaatg cccaagagat 1680
gatgcttgta caaggaaact ttactttttt gttgcaatac aagtcttgaa tttatttttc 1740
tctgcacttg gaggcacctc acatgtcatg ctgattgtta aaattgttca acctgaattg 1800
aaatcaactg cactgggttt ccaactcaatg gttatacagag cactaggagg aattctagct 1860
ccaatatatt ttggggctct gattgataca acgtgtataa agtgggtccac caacaactgt 1920
ggcacacgtg ggtcatgtag gacatataat tcacatcat tttcaagggt ctacttgggc 1980
ttgtcttcaa tgttaagagt ctcacactt gttttatata ttatattaat ttatgccatg 2040
aagaaaaaat atcaagagaa agatatcaat gcacagaaa atggaagtgt catggatgaa 2100
gcaaaccttag aatccttaaa taaaaataaa cattttgtcc ctctgtctgg ggcagatagt 2160
gaaacacatt gttaaggggg gaaaaaaagc cacttctgct tctgtgtttc caaacagcat 2220
tgcatttgatt cagtaagatg ttatttttga ggagttcctg gtcctttcac taagaatttc 2280
cacatctttt atggttgaag tataaataag cctatgaact tataataaaa caactgttag 2340
gtagaaaaaa tgagagtact cattgtacat tatagctaca tatttgttgt taagggttaga 2400
ctatatgatc catacaaatt aaagttagag acatgggttac tgtgtaataa aa 2452

```


EP 1 186 672 A2

<210> 2
 <211> 691
 <212> PRT
 <213> Homo sapiens

5

<400> 2
 Met Asp Gln Asn Gln His Leu Asn Lys Thr Ala Glu Ala Gln Pro Ser
 1 5 10 15

10

Glu Asn Lys Lys Thr Arg Tyr Cys Asn Gly Leu Lys Met Phe Leu Ala
 20 25 30

Ala Leu Ser Leu Ser Phe Ile Ala Lys Thr Leu Gly Ala Ile Ile Met
 35 40 45

15

Lys Ser Ser Ile Ile His Ile Glu Arg Arg Phe Glu Ile Ser Ser Ser
 50 55 60

Leu Val Gly Phe Ile Asp Gly Ser Phe Glu Ile Gly Asn Leu Leu Val
 65 70 75 80

20

Ile Val Phe Val Ser Tyr Phe Gly Ser Lys Leu His Arg Pro Lys Leu
 85 90 95

Ile Gly Ile Gly Cys Phe Ile Met Gly Ile Gly Gly Val Leu Thr Ala
 100 105 110

25

Leu Pro His Phe Phe Met Gly Tyr Tyr Arg Tyr Ser Lys Glu Thr Asn
 115 120 125

Ile Asn Ser Ser Glu Asn Ser Thr Ser Thr Leu Ser Thr Cys Leu Ile
 130 135 140

30

Asn Gln Ile Leu Ser Leu Asn Arg Ala Ser Pro Glu Ile Val Gly Lys
 145 150 155 160

Gly Cys Leu Lys Glu Ser Gly Ser Tyr Met Trp Ile Tyr Val Phe Met
 165 170 175

Gly Asn Met Leu Arg Gly Ile Gly Glu Thr Pro Ile Val Pro Leu Gly
 180 185 190

35

Leu Ser Tyr Ile Asp Asp Phe Ala Lys Glu Gly His Ser Ser Leu Tyr
 195 200 205

Leu Gly Ile Leu Asn Ala Ile Ala Met Ile Gly Pro Ile Ile Gly Phe
 210 215 220

40

Thr Leu Gly Ser Leu Phe Ser Lys Met Tyr Val Asp Ile Gly Tyr Val
 225 230 235 240

Asp Leu Ser Thr Ile Arg Ile Thr Pro Thr Asp Ser Arg Trp Val Gly
 245 250 255

45

Ala Trp Trp Leu Asn Phe Leu Val Ser Gly Leu Phe Ser Ile Ile Ser
 260 265 270

Ser Ile Pro Phe Phe Phe Leu Pro Gln Thr Pro Asn Lys Pro Gln Lys
 275 280 285

50

Glu Arg Lys Ala Ser Leu Ser Leu His Val Leu Glu Thr Asn Asp Glu
 290 295 300

Lys Asp Gln Thr Ala Asn Leu Thr Asn Gln Gly Lys Asn Ile Thr Lys
 305 310 315 320

55

Asn Val Thr Gly Phe Phe Gln Ser Phe Lys Ser Ile Leu Thr Asn Pro
 325 330 335

EP 1 186 672 A2

	Leu Tyr Val Met Phe Val Leu Leu Thr Leu Leu Gln Val Ser Ser Tyr	
	340 345 350	
5	Ile Gly Ala Phe Thr Tyr Val Phe Lys Tyr Val Glu Gln Gln Tyr Gly	
	355 360 365	
	Gln Pro Ser Ser Lys Ala Asn Ile Leu Leu Gly Val Ile Thr Ile Pro	
	370 375 380	
10	Ile Phe Ala Ser Gly Met Phe Leu Gly Gly Tyr Ile Ile Lys Lys Phe	
	385 390 395 400	
	Lys Leu Asn Thr Val Gly Ile Ala Lys Phe Ser Cys Phe Thr Ala Val	
	405 410 415	
15	Met Ser Leu Ser Phe Tyr Leu Leu Tyr Phe Phe Ile Leu Cys Glu Asn	
	420 425 430	
	Lys Ser Val Ala Gly Leu Thr Met Thr Tyr Asp Gly Asn Asn Pro Val	
	435 440 445	
20	Thr Ser His Arg Asp Val Pro Leu Ser Tyr Cys Asn Ser Asp Cys Asn	
	450 455 460	
	Cys Asp Glu Ser Gln Trp Glu Pro Val Cys Gly Asn Asn Gly Ile Thr	
	465 470 475 480	
25	Tyr Ile Ser Pro Cys Leu Ala Gly Cys Lys Ser Ser Ser Gly Asn Lys	
	485 490 495	
	Lys Pro Ile Val Phe Tyr Asn Cys Ser Cys Leu Glu Val Thr Gly Leu	
	500 505 510	
30	Gln Asn Arg Asn Tyr Ser Ala His Leu Gly Glu Cys Pro Arg Asp Asp	
	515 520 525	
	Ala Cys Thr Arg Lys Phe Tyr Phe Phe Val Ala Ile Gln Val Leu Asn	
	530 535 540	
35	Leu Phe Phe Ser Ala Leu Gly Gly Thr Ser His Val Met Leu Ile Val	
	545 550 555 560	
	Lys Ile Val Gln Pro Glu Leu Lys Ser Leu Ala Leu Gly Phe His Ser	
	565 570 575	
40	Met Val Ile Arg Ala Leu Gly Gly Ile Leu Ala Pro Ile Tyr Phe Gly	
	580 585 590	
	Ala Leu Ile Asp Thr Thr Cys Ile Lys Trp Ser Thr Asn Asn Cys Gly	
	595 600 605	
45	Thr Arg Gly Ser Cys Arg Thr Tyr Asn Ser Thr Ser Phe Ser Arg Val	
	610 615 620	
	Tyr Leu Gly Leu Ser Ser Met Leu Arg Val Ser Ser Leu Val Leu Tyr	
	625 630 635 640	
	Ile Ile Leu Ile Tyr Ala Met Lys Lys Lys Tyr Gln Glu Lys Asp Ile	
	645 650 655	
50	Asn Ala Ser Glu Asn Gly Ser Val Met Asp Glu Ala Asn Leu Glu Ser	
	660 665 670	
	Leu Asn Lys Asn Lys His Phe Val Pro Ser Ala Gly Ala Asp Ser Glu	
	675 680 685	
55	Thr His Cys	

EP 1 186 672 A2

690

5
<210> 3
<211> 1538
<212> DNA
<213> Homo sapiens

<400> 3
10 atgctctttt acctctgaaa atattggaga attttacaac tggcaccttt agctcaggat 60
tataaagggt gttagttagt ttgtactgtt ttatcttcat tgtatataat atatataatta 120
gtctccaaac atgttgatgt gttttcaatg aaatggatgt ctgaggagaa aaccattagc 180
ctgagaaaaa ccaaaactgta ttcccatgtt gaataaaagg aagtccataa aaatgatgga 240
aaatgttctg cattcctgtt atgatatcaa aatctggcag tacatgaaaa tttttcaaag 300
tgcttattta acaggcataa tctttggtct cctgagccag aatctgctgg gtatgggact 360
ggattgctat tttgacaact cgccagtaga ttcttactca gcagagtatt tgggaagcctt 420
15 actctaatat ttggccttg ggtctacatt tctcagttct gcacagtcatt tcttccctc 480
tacactactc tttagtttgt ctcatgattc caatactctc aataattaac caagaataga 540
actaatcaat cagataaactg tggcacagac atcaaataca ttttgctgca accatatcaa 600
caaatgtccc atgaatgata aggggtaacc atattctcat atatgcatcc tcacattacc 660
acatatatat atgtgcatat gtgtatacag gtaaaagtgt gtatatatgt atacatgtat 720
gtttgtgtgt atatacatat atatatcttc acacttttct gaaatatata tatttatgag 780
20 agagaagggt ctgtacttta tttcagaaga gagcttaatg tccaaggtat aattgagagt 840
ctaaaatggt tgagttattg aattaattaa acttcatctc tactcaagaa aacttttaac 900
tgagtttaag tcttcttttc tccacaagtc aagtcaataa aaggaaactg tgatattaat 960
aattcttttc tgttttgatg taaagaatct atcgcataaa gcagtcttaa ttttcatcat 1020
tcagaaaaat ggtcttgtag ttaattggga ctctcttatt ccagggtggt tctccagttc 1080
ccatacatat cactgttagaa ccatacttat gtaccaagca aagagggtat attttaattt 1140
25 ttaaatgcca atgtaacctg taggcatatt ttttatttgt cttaaattat ttcctatttg 1200
gaagttttaa atacctggaa taattttatt tactcatatt tttaaagaaa aaaatcttat 1260
gccaccaact taattgaata aacaagtaaa agccattccc aaaagtaagg tttacttggt 1320
aagatttaaca aaaaataatg tgagaattct gagaaatata atctttaaat attggcaact 1380
ggagtgaact cttaaaacta actagggttt atatgtttga ctgagcaat gacataataa 1440
gggtggttaat catcactgga cttgttttca aaaagccaac tactttaaga ggaataaagg 1500
30 gtggacttgt tgcagttgct gtaggattct aaatccag 1538

35
<210> 4
<211> 200
<212> DNA
<213> Homo sapiens

<400> 4
40 gataactgca tggattgaag ctagaataag ttttatgttt ttgagctaaa ataagtaaat 60
agggaaacttt aatgtataga aaagcaagt gttaaaaaga acattatggt tcaaattata 120
attttcaatt gaagcatata ttgaaatatt aacataatga ttcatacctt gattttaaacc 180
agtcttttaa tctgattaag 200

45
<210> 5
<211> 300
<212> DNA
<213> Homo sapiens

<400> 5
50 tgacggaagc tttgaaattg gtaacattta ttttctattt taataaccaa acttgcaaag 60
ttaaaaaata tatatgcttt acaccactgg ttatcaactg gggtaaattt atctctcaca 120
ggcaatttgg caataactaa aaacatttgt ggttgtcata actgcacagg ggttgggggc 180
aatggaagtg ctactggtat ctaaaaggtag aggtcagggg tactgctaaa tattctataa 240
55 tgcacaaaaga atgatgtaac tgaaaatggt gatagtgagg atgttcagaa accctgattc 300

55
<210> 6
<211> 300
<212> DNA
<213> Homo sapiens

<400> 6

EP 1 186 672 A2

ccacatttct tcatgggata gtaagtgtta aaaaaaaaaa aaacctctgt gccactatca 60
 gtaccttgta aattaggagt agaattttat tattatccct ttaaataggc agttaccttt 120
 tgagaagata cccactaagt gtgtacagaa atgaaatagt gtctatttgt ctacataatc 180
 attttattta tcgtagcttt catatacttt gaaataacaa aaagactaaa ctgtagagtt 240
 5 tcaaatgaaa taaataggct ttttatgaat ttttagtata acgtatatac tgtacgtctt 300

<210> 7
 <211> 300
 <212> DNA
 10 <213> Homo sapiens

<400> 7
 acctgagata gtgggaaaag gtaagaatta atattgacag taaaaagtct tctaaaatgt 60
 atacatttaa ttacatctct aaaaattggt gtgatattca ttagcaaaat ttaattaaga 120
 atgaatagga aaaacatttg actcttacag acataattat agtggttaata tacacagtcc 180
 15 gccattaac aacacagggt taaactacgc gttttcactt ctatgcaaat ttgtccatc 240
 tgaactggat gataaacctg ccggtaaaga tatctgacat tttctatat tggattgaac 300

<210> 8
 <211> 200
 <212> DNA
 20 <213> Homo sapiens

<400> 8
 tagcagcata agaatggact aatacaccat attgtcaaag ttgcaaaagt gaatataaat 60
 tacttgtact tgtaaattaa aaaaaaataa gtagaataat taagagttta caagtagtta 120
 aatttgtaat agaaatgcta aaattaatgt ttaaaatgaa acactctctt atctacatag 180
 25 gttgttttaa ggaatctggg 200

<210> 9
 <211> 200
 <212> DNA
 30 <213> Homo sapiens

<400> 9
 tattggatgc gtagatctaa gtaagtacaa ccagaacaag gtaccatgat aacgtctttc 60
 taagcacaca tgcgaaaaac attttttcaa ataactgaat tcaactcttc aatagtcctt 120
 tgcttaatat aattagaaag ttacaagtag gaaataaatg tattactaat cagaataaat 180
 35 ataaaatcca gctcctatct 200

<210> 10
 <211> 203
 <212> DNA
 40 <213> Homo sapiens

<400> 10
 ttaaaaaaaa ctttgccatt tcgtcatcat caaagcaaat ttcttcatat aaagaaaaat 60
 tctttatcta ctttttcttt tccctctttc tctgctttca ctttacttct tcttctctct 120
 ccccttcttt gtctttttct tctctctctc tctttttgat atatgtctat catatatctc 180
 45 cagaaataat ccagtgcacat ctc 203

<210> 11
 <211> 201
 <212> DNA
 50 <213> Homo sapiens

<400> 11
 catgtcatgc tgattgttaa gtaagtatga cttttaaaaa cattttcata tgcattgagc 60
 tataaacaca cctaagtata tgcataattt tacataatat actgggaatt caaattcata 120
 tttcatcaaa ttttaatttt ctgagaattc attttattaa aatttactat gaactctcaa 180
 55 ggctgtaatt aataattttg c 201

EP 1 186 672 A2

<210> 12
<211> 200
<212> DNA
<213> Homo sapiens

<400> 12
tgatttgggt ctttgagatt tetaataatc tttattattg ggtagatgca gaacaaaata 60
ataaacgaat cctccaaatt tttgaacttt tatttaataca aaatatatca atgtggaata 120
tcatgcagtt acatttataa tatgttcocct aaactgacat cttctcttct cctattacag 180
gaggaattct agctccaata 200

Claims

1. A method for the detection of a polymorphism in OATPC in a human, which method comprises determining the sequence of the human at at least one of the following polymorphic positions:

positions 510, 696, 1299, 1312, 1347, 1561, 2028, 2327 and 2342 in sequence of the OATPC gene as defined by the position in SEQ ID NO: 1;
positions 400, 405, 488 and 643 in OATPC polypeptide defined by position in SEQ ID NO: 2;
positions 321 and 1332 defined by position in SEQ ID NO 3;
position 41 defined by position in SEQ ID NO 4;
positions 109 and 244 defined by position in SEQ ID NO 5;
positions 117 and 283 defined by position in SEQ ID NO 6;
positions 209 and 211 defined by position in SEQ ID NO 7;
positions 63 to 68 defined by position in SEQ ID NO 8;
position 53 defined by position in SEQ ID NO 9;
position 75 defined by position in SEQ ID NO 10;
position 162 defined by position in SEQ ID NO 11; and
positions 84 defined by position in SEQ ID NO 12.

2. Use of a method as defined in claim 1 to assess the pharmaco genetics of a drug transportable by OATPC.

3. A polynucleotide comprising at least 20 bases of the human OATPC gene and comprising an allelic variant selected from any one of the following:

Region	variant	Position in SEQ ID NO	SEQ ID NO
Exon 4	A	510	1
Exon 5	T	670	1
Exon 5	T	696	1
Exon 9	G	1299	1
Exon 9	A	1312	1
Exon 9	A	1347	1
Exon 10	C	1561	1
Exon 14	C	2028	1
3'UTR	Insert T	2327	1
3'UTR	C	2342	1
Promoter	G	321	3
Promoter	C	1332	3
Intron 1	A	41	4

EP 1 186 672 A2

(continued)

Region	variant	Position in SEQ ID NO	SEQ ID NO
Intron 2	G	109	5
Intron 2	G	244	5
Intron 3	A	117	6
Intron 3	A	283	6
Intron 4	A	209	7
Intron 4	A	211	7
Intron 4	Deletion CTTGTA	63	8
Intron 6	T	53	9
Intron 9	Insert TTC	75	10
Intron 11	Insert T	162	11
Intron 12	C	84	12

4. A nucleotide primer which can detect a polymorphism as defined in claim 1.
5. An allele specific primer capable of detecting a OATPC gene polymorphism as defined in claim 1.
6. An allele-specific oligonucleotide probe capable of detecting a OATPC gene polymorphism as defined in claim 1.
7. Use of an OATPC polymorphism as defined in claim 1 as a genetic marker in a linkage study.
8. Use of a drug transportable by OATPC in preparation of a medicament for treating a disease in a human detected as having a OATPC polymorphism at one or more of the following positions:

positions 487, 510,, 554, 670, 696, 819, 820, 1299, 1312, 1347, 1561, 2028, 2327 and 2342 in sequence of the OATPC gene as defined by the position in SEQ ID NO: 1;
positions 130, 152, 174, 241, 400, 405, 488 and 643 in OATPC polypeptide defined by position in SEQ ID NO: 2;
positions 321 and 1332 defined by position in SEQ ID NO 3;
position 41 defined by position in SEQ ID NO 4;
positions 109 and 244 defined by position in SEQ ID NO 5;
positions 117 and 283 defined by position in SEQ ID NO 6;
positions 209 and 211 defined by position in SEQ ID NO 7;
positions 63 to 68 defined by position in SEQ ID NO 8;
position 53 defined by position in SEQ ID NO 9;
position 75 defined by position in SEQ ID NO 10;
position 162 defined by position in SEQ ID NO 11; and
positions 84 defined by position in SEQ ID NO 12.

9. A use according to claim 10 in which the drug is a statin.
10. A use according to claim 10 in which the drug is rosuvastatin.
11. An allelic variant of human OATPC polypeptide comprising at least one of the following:
 - a leucine at position 400 of SEQ ID NO 2;
 - an isoleucine at position 405 of SEQ ID NO 2;
 - an arginine at position 488 of SEQ ID NO 2;
 - a phenylalanine at position 643 of SEQ ID NO 2;

or a fragment thereof comprising at least 10 amino acids provided that the fragment comprises at least one allelic

variant.

12. An antibody specific for an allelic variant of human OATPC polypeptide as defined in claim 11.

5

10

15

20

25

30

35

40

45

50

55